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MINI REVIEW

Regulation of T cell activation in Sjögren's syndrome

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Summary Autoimmune diseases reveal complex and multigenic phenotypes affected by a variety of genetic and environmental or stochastic factors. A lot of models for autoimmune diseases including Sjögren's syndrome (SS) have been demonstrated using gene-manipulated or other animals. Previous findings obtained with both animal models and patients with SS indicate involvement of a T cell with a T helper type 1 (T_H1) phenotype. This review focuses on the T cell-dependent autoimmune disease such as SS to understand the molecular mechanism of regulation of T cell activation in autoimmunity, and highlights the pathogenesis of autoimmune disease.

1. Introduction

Our body is protected by precise immune system consisted of a number of immune cells which play each roles in maintenance for various immune responses. Among them, T cells play a central role in both cellular and humoral immunity [1]. After antigenic stimulation, naïve T cells proliferate and differentiate into various effector subsets characterized by the production of distinct cytokines and by their distinct effector functions [2]. Although almost autoimmune diseases occur through the pathogenesis based on $CD4^+$ T cell dysfunction or failure of differentiation in the thymus or peripheral lymphoid organs, the precise mechanism how autoreactive T cell emerges has been still unclear. In this review, we outline current information as to the association of T cell regulation with autoimmunity, and indicate the molecular mechanism for pathogenesis of SS, an organ-specific autoimmunie disease in salivary glands.

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2. T cell-dependent autoimmunity

The $CD4^+$ T cells were designated T_H1 cells, characterized by the secretion of interleukin (IL)-2 and interferon (IFN)-γ but not IL-4 and IL-5, and T helper type 2 (T_H2) cells, which were characterized by the secretion of IL-4 and IL-5 but not IL-2 or IFN-γ (Fig. 1). T_H1 cells elicit delayed-type hypersensitivity responses, activate macrophages and are highly effective in clearing intracellular pathogens [3]. By contrast, T_H2 cells are crucial for the production of immunoglobulin E and eosinophilic inflammation such as allergic diseases and may suppress cell-mediated immunity [4]. Besides, several subsets of regulatory T (T_{reg}) cells capable of controlling effector T cells have been well known [5]. Furthermore, recent reports have demonstrated that T helper 17 (T_H17) cells as a new subset produce IL-17, and are involved in the various immune responses [6]. Dysregulated or uncontrolled effector T cell responses lead to autoimmune disease, suggesting that breakdown of central or peripheral tolerance induces dysfunction of T cell subsets and then the onset of any autoreactive response.

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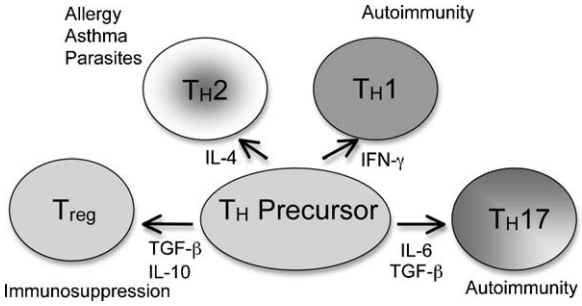

Figure 1 Differentiation of $CD4^+$ T cell lineages. Peripheral naïve $CD4^+$ T cell precursor cells can differentiate into three subsets of effector T cells and several subsets of T_{reg} cells. Each subset is associated with the pathogenesis of many immune disorders.

T_H1 cells are highly proinflammatory and have been linked to the induction and progression of a lot of autoimmune diseases in human or animal models [4,7,8]. On the other hand, it has been reported that T_H17 cells play an important role in the pathogenesis of autoimmune diseases such as rheumatoid arthritis (RA), multiple sclerosis, systemic lupus erythematous (SLE) and asthma [6]. Moreover, it is well known that natural $CD25^+CD4^+$ T_{reg} cells are one of key subsets for inducing or attenuating immunological tolerance to self or non-self antigens [5]. For instance, depletion or dysfunction of natural $CD25^+CD4^+$ T_{reg} cells suffices to cause autoimmune disease in normal mice by administration of neutralizing antibody against CD25 molecule [9]. Also, preventive or therapeutic effects by adoptive transfer of $CD25^+CD4^+$ T_{reg} cells into autoimmune models were demonstrated in previous reports [10]. Thus, the T cell-dependent autoimmune diseases are occurred based on the breakdown of proportion or function of T cell subsets in the immune system.

SS is an autoimmune disorder characterized by lymphocytic infiltrates and destruction of salivary and lacrimal glands (Fig. 2), leading to clinical symptoms of dryness of the mouth and eyes (sicca syndrome) which may predispose patients to oral or ocular infections [11]. In the salivary gland tissues from SS patients, predominance of $CD4^+$ T cells in addition to a small number of $CD8^+$ T cells, B cells, or macrophages is observed. It is well known that T_H1 cells producing IL-2 and IFN-γ play a central role in the onset of both human SS and the animal models [7,8,12,13].

3. Autoimmune models

Why do self-T cells attack self-tissues, cells or antigens? Analyzing animal models is useful to understand the pathogenesis or mechanism of human autoimmune disease. The three types of animal models for autoimmune diseases are known. The first is the type of autoimmune prone animals that any autoimmune diseases are naturally appeared, including NOD mouse of a model for autoimmune diabetes (type I DM), MRL/*lpr* mouse of a model for RA and SLE [14,15]. These mice have often been used for analyzing the pathogenic mechanism as the lesions are naturally occurred, and the incidence of disease onset is relatively constant compared with the other models. The second is drug-induced or operation-induced models such as the autoimmune model in multiorgans by injection of neutralizing antibody against CD25 as mentioned above or a murine SS model by neonatal thymectomy for NFS/*sld* mouse [6,12]. Although the handling or manipulating these models is difficult, it is useful to analyze the autoimmune lesions during short term. The third is a type of gene-manipulated animal including any gene knockout (KO) or transgenic (TG) mice. These models are helpful to define the molecular mechanism of autoimmune diseases.

For instance, a number of murine models showing SS-like autoimmune lesions have been reported as shown in Table 1. The (NZB/NZW)F1 mouse was one of the first models of spontaneous SS [16]. NOD and MRL/*lpr* mice have been described to be models for the secondary SS besides the lesions of autoimmne diabetes, or RA and SLE [14,15]. The secondary SS in human is also known to accompany with RA, SLE, or the other autoimmune diseases [11]. Additionally, although *aly*/*aly* mouse bearing a mutant of NF-κB-inducing kinase (NIK) gene has been reported to be an SS model, autoimmune lesions are observed in multiorgans other than salivary glands [17]. As a model for the primary SS, the NFS/*sld* mouse thymectomized on 3-day after birth has been established [12]. We have analyzed the model to detect

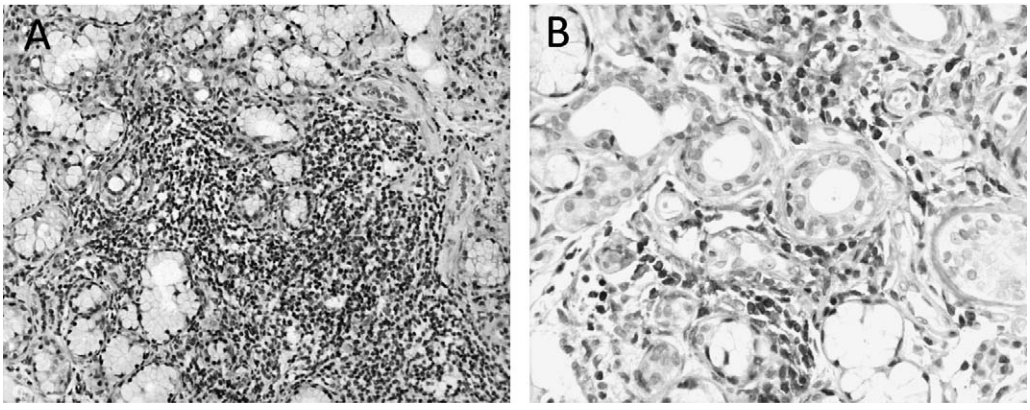

Figure 2 Pathology of salivary gland tissues from SS patients. A Lymphocytic infiltration with tissue destruction in salivary gland from SS patients was observed. Lip biopsy materials were used for staining with hematoxylin and eosin. A representative photo is shown. B Immunohistochemical staining of $CD3^+$ T cells was performed using the paraffin-embed sections of salivary glands from SS patients.

Table 1 Representative murine models for human SS.

Year [Ref. no.]	Mouse	Related data
1979 [16]	NZB/NZW F1	Ro60, IFN-γ
1983 [14]	MRL/*lpr*	Fas, RA, SLE
1989 [27]	*HTLV-1 tax* TG	Human T cell leukemia
1992 [15]	NOD	Type I DM
1994 [12]	NFS/*sld*	thymectomy, α-fodrin
1996 [20]	*TGF-β1* KO	IFN-γ
1996 [17]	*aly/aly*	NIK
1999 [21]	*IL-10* TG	FasL
2002 [23]	*AIRE* KO	autoantigens
2004 [29]	*Aromatase* KO	α-fodrin
2004 [25]	*Id3* KO	T cell development
2006 [26]	*ClassIA PI3K* KO	SSA, IFN-γ, Treg
2006 [22]	*IL-14α* TG	Aging, $CD5^+$B cell
2006 [24]	*CCR7* KO	thymic T cell selection
2006 [28]	*NZW2328*	MCMV
2008 [30]	RbAp48 TG	estrogen

one of salivary gland-specific autoantigens for SS and the antigen-specific response of T_H1 cells, and define the breakdown of peripheral tolerance by dysfunction of activation-induced cell death (AICD) that is a system to delete autoreactive T cells in the periphery [18,19]. Besides, several KO mice have been reported that SS-like lesions are observed in salivary or lacrimal glands. Cytokine gene-manipulated mice such as *TGF-β1* KO, *IL-10* TG, or *IL-14α* TG mice have been reported to be spontaneous SS models [20—22]. Also, the SS-like lesions have been indicated in some KO mice for autoimmune regulator (AIRE) [23], a transcription factor as known to be important for the expression of autoantigen in thymus, or C-C chemokine receptor 7 (CCR7), a chemokine receptor for CCL19 and CCL21, which plays a crucial role in the T cell selection in thymic medulla [24]. Also, in the KO mice for Id3 and class IA phosphoinositide-3-kinase (PI3K) which are related to differentiation or function of immune cells including T cells, inflammatory lesions of salivary or lacrimal glands were reported [25,26]. On the other hand, SS-like lesions were induced by transgene of virus-related antigen or virus infection [27,28]. As for environmental factor such as hormone, autoimmne lesions of salivary glands from *aromatase* KO mice are observed [29]. Recently, we have established retinoblastoma-associated protein 48 (RbAp48) TG mouse as one of SS models in which transgenic expression of RbAp48 in the salivary glands resulted in the development of autoimmune exocrinopathy resembling SS [30]. These studies using several SS models raise the new findings as to not only the pathogenesis of SS, but also the molecular mechanisms for central or peripheral T cell tolerance in the immune system.

4. Transcription factors in pathogenesis of autoimmunity

What is happening to the inside of T cell in autoimmune response? Several transcription factors controlling target genes essential for cell survival, cytokine production, chemokine production, adhesion molecule expression, organogenesis, and apoptosis play central roles in T cell activation [31]. Among them, nuclear factor (NF)-κB plays a key role in regulating many inflammatory processes of T cells [32,33]. NF-κB has five subunits including NF-κB1 (p50 and its precursor p105), NF-κB2 (p52 and its precursor p100), RelA (p65), RelB, and c-Rel.

Antigenic stimuli from T cell receptor (TCR), co-stimulatory molecules such as CD28, and cytokine receptors leads to NF-κB activation through a lot of signal molecules. Two main pathways of NF-κB are well known. One is the classical pathway (canonical pathway) through complex of NF-κB1 and RelA [34,35]. The other pathway is through complex of NF-κB2 and RelB (non-classical pathway, non-canonical pathway, or alternative pathway) [34,35]. Signals from TCR and CD28 through many adaptor molecules lead to protein kinase C (PKC) activation. Downstream of PKC, there is CARMA1/MALT1/Bcl-10, which activate IκB kinase (IKK). IKK phosphorylates IκB, which binds to NF-κB complex in the cytoplasm to inhibit nuclear translocation. Ubiquitination and degradation of IκB can induce transport of NF-κB complex into the nucleus. As a result, NF-κB complexes control transcription of the target genes essential for T cell activation. Also, signals from TCR/CD28 or cytokine receptors lead to PKC, NIK, and CARMA1/MALT1/Bcl-10. Activation of IKK processes p100 into p52, and complex of p52 and RelB is translocated into the nucleus to regulate transcription of the target genes. It has been reported that the classical pathway of NF-κB is essential for innate immunity [36,37]. Moreover, many data suggest that the alternative pathway plays a central role in the expression of genes involved in development and maintenance of secondary lymphoid organs [35]. Non-classical pathway of NF-κB has been described that is independent of IKKγ and involves the processing of the p100 precursor to p52, which is triggered by the phosphorylation of p100 by the NIK and IKKα [36,37]. In addition, the importance of several signal molecules for NF-κB activation including NIK, IKKs, and IκBs has been highlighted with several mouse models [31]. The most important phenotype for *aly/aly,* and *NIK* KO mice is autoimmune disease in multiorgans including lacrimal gland, salivary gland, lung, liver, kidney, and pancreas [17].

Our study demonstrates that proliferative response of naïve $CD4^+$ T cells from *aly/aly, NIK* KO, *RelB* KO mice, but not memory T cells, is higher than that from control mice, suggesting that NF-κB2 deficiency might result in the hyperproliferation of naïve T cells to respond to any self-proteins to induce autoimmune disease [38].

The processing of NF-κB2/p100 to p52 is controlled by a kinase cascade including activation of IKKα through NIK. Based on our research, the main NF-κB complex p50-RelA for normal naïve $CD4^+$ T cell activation might be retained in the cytoplasm to interact with p100 [38]. It is possible that over-activation of naïve $CD4^+$ T cells would be regulated by the interaction between NF-κB1 (p50)-p65 and NF-κB2 (p100) to inhibit nuclear translocation of p50-p65 dimmers as shown in Fig. 3. This possibility corresponds to a previous data which accumulated p100 of activated osteoclasts from *NIK* KO mice would bind NF-κB1-RelA subsets to prevent their nuclear transport and inhibit osteoclastogenesis [39].

When naïve T cells of *aly/aly* mice were transferred into *Rag2* KO mice which have no T and B cells in the periphery, the severe autoimmune lesions in lacrimal glands, lung, and salivary glands were observed [38]. Therefore, it is possible that NF-κB2-deficient T cells may be hyperreactive to any self-antigen through excessive activation of NF-κB1/RelA

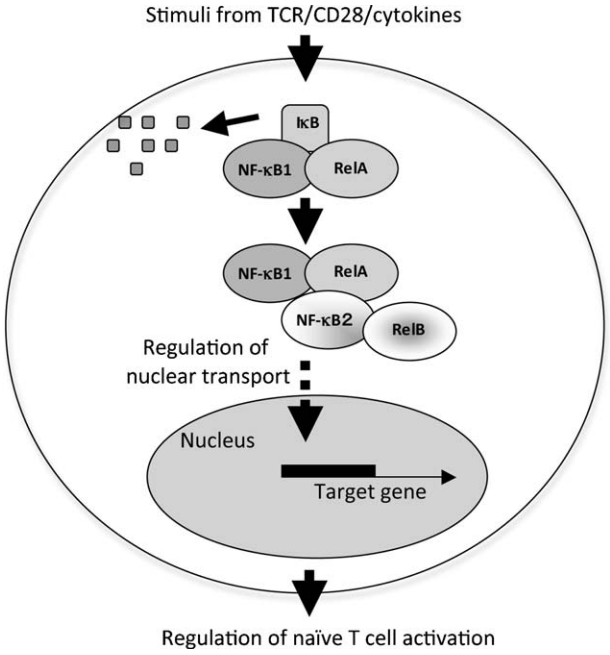

Figure 3 Novel regulation mechanism of NF-κB by the non-classical NF-κB pathway. Stimuli from T cell receptor (TCR), CD28, or cytokine receptors lead to phospolyration, ubiqutination, and degradation of IκB following nuclear transport of NF-κB1/RelA complex. After regulation by IκB, NF-κB2/RelB complex binds to the NF-κB1/RelA complex to control the translocation into the nucleus.

pathway, suggesting that NF-κB2 is one of key molecules for T cell regulation in autoimmunity.

5. Conclusion

Based on previous reports as mentioned above, T_H1 cells play a central role in the pathogenesis for SS in human and the animal models. Our studies and the other reports have demonstrated that apoptosis of Fas-expressing salivary gland cells stimulated by cytokines which T_H1 cells secreted was undergone by interaction with Fas ligand (FasL)-expressing activated T cells [19,21]. However, it is still unclear why salivary or lacrimal glands are attacked as a target organ. The breakdown of T cell tolerance via disorders of signaling molecules for T cell activation such as NF-κB may tend to induce autoimmune lesions in salivary and lacrimal glands resembling human SS, suggesting that salivary or lacrimal gland cells are much more sensitive to any cytokines or stimulation factors from autoreactive and pathogenic T cells. We hypothesize that there may be a unique system ''local immune tolerance'' in salivary and lacrimal glands [30]. With the loss of local immune tolerance by any change of immune homeostasis, the salivary and lacrimal gland cells might become target cells toward autoreactive T cells. It is possible that there are complex mechanisms including expression of autoantigen, MHC class II, or any molecules related with autoimmunity of the target cells for triggering autoimmune disease.

Considerable progress has been made in elucidating the immunological events surrounding autoimmune disease. Despite such progress, however, a lot of important critical issues remain to be addressed. Additional advances will come from further studies of experimental models or human patients to address specific aspects of the disease.

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